Distinct Intracellular Mouse Thyrotropin-Releasing Hormone Receptor Domains Determine Subtype-Specific G-protein Coupling Preferences

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The two mouse thyrotropin-releasing hormone (TRH) receptor subtypes (mR1 and mR2) exhibit indistinguishable binding affinities for TRH but differences in signaling and internalization properties. mR2 has a significant TRH-independent signaling activity compared to mR1. For both receptors, intracellular signal transduction is mediated primarily by coupling to Gq/G11 proteins, but under specific conditions in certain cell types activation of Gi-2 and Gi-3 by TRH-R1 was also observed. We have identified differences between mR1 and mR2 in G-protein activation preferences using AP-1- and CREB-responsive reporter genes in transiently transfected HEK 293 EM cells. The effects of mR1 and mR2 signaling on activation of CREB activity were comparable to the activation of the inositol phosphate pathway. This signaling was not affected by pertussis toxin (PTX) suggesting both responses are mediated by Gq/11 activation because coupling to Gi/o would be inhibited by PTX. In contrast, PTX reduced by 40% TRH-stimulated activation of AP-1 by mR1, but had no effect on the basal or TRH-stimulated activation of AP-1 by mR2. Therefore, only TRH-stimulated mR1 regulation of AP-1 activity appeared to be mediated by Gi/Go. The carboxyl-terminus and intracellular loops (ICLs) of G-protein coupled receptors have been shown to play a key role in G-protein activation. To gain insight into the contribution of the intracellular domains of mR1 on Gi/Go-mediated activation of AP-1, we used chimeric receptors in which the individual ICLs or the carboxyl terminal tail of mR1 were replaced with those of mR2. PTX treatment of the cells inhibited TRH-stimulated AP-1 activity for all chimeric constructs except mR1 with ICL2 of mR2. To investigate whether ICL2 of mR1 is sufficient to confer Gi/Go coupling and PTX sensitivity to mR2, we generated a chimeric receptor in which ICL2 of mR2 was replaced with that of mR1. AP-1 signaling was not affected by PTX in cells transfected with this mutant and signaling behavior was comparable to wild type mR2. Our data provide evidence that mR1, but not mR2, signals through a PTX-sensitive pathway leading to activation of AP-1 and that ICL2 of mR1 appears to be involved in this subtype-specific Gi/Go activation but is not sufficient alone. These data provide further evidence for important differences in signaling properties between mR1 and mR2.